Empirical Bayes Procedure for Estimating Genetic Distance Between Populations and Effective Population Size

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ABSTRACT

We developed an empirical Bayes procedure to estimate genetic distances between populations using allele frequencies. This procedure makes it possible to describe the skewness of the genetic distance while taking full account of the uncertainty of the sample allele frequencies. Dirichlet priors of the allele frequencies are specified, and the posterior distributions of the various composite parameters are obtained by Monte Carlo simulation. To avoid overdependence on subjective priors, we adopt a hierarchical model and estimate hyperparameters by maximizing the joint marginal-likelihood function. Taking advantage of the empirical Bayesian procedure, we extend the method to estimate the effective population size using temporal changes in allele frequencies. The method is applied to data sets on red sea bream, herring, northern pike, and ayu broodstock. It is shown that overdispersion overestimates the genetic distance and underestimates the effective population size, if it is not taken into account during the analysis. The joint marginal-likelihood function also estimates the rate of gene flow into island populations.

As a stock management tool to counteract decreased or depleted fishery resources, stock enhancement programs have been undertaken in many countries for salmonid (Hilborn and Winton 1993; Ritter 1997; Kaeriyama 1999; Knapp 1999) and for other marine species (Bartley 1999; Kitada 1999). Concerns about the genetic effects of hatchery releases on wild populations have increased and aroused discussion (Walters 1988; Waples 1991; Hilborn 1992; Utter 1998; Waples 1999). Campton (1995) reviewed the genetic effects of hatchery releases on natural stocks of salmon and brown trout and concluded that the empirical data supporting those arguments are absent or largely circumstantial. This is a complex topic that needs further research (Waples 1999). A 10-point approach for a responsible stock enhancement program has been proposed, which includes the need to use genetic resource management to avoid deleterious genetic effects (Blankenship and Leber 1995). Using wild individuals as broodstock may possibly reduce genetic risks (Bartley *et al.* 1995; Harada *et al.* 1998).

The genetic identity between produced progenies and the wild stock will be required before one can release the progenies. To examine the genetic identity, statistically significant differences are required. The homogeneity χ^2 test of allele frequencies is commonly used for testing genetic differences and the Roff test (Roff and Bentzen 1989) is used when minor alleles exist. If the null hypothesis is not rejected, the statistical power is required to be reported from a conservation viewpoint (Peterman 1990; Dizon *et al.* 1995). However, when the genetic difference is small, the corresponding statistical power may also be small with small sample sizes, making it difficult to conclude that there is no genetic difference. The statistical power is the probability of detecting the alternative hypothesis when it is correct. A considerably large sample size is required if one wants to obtain satisfactory large statistical power to reject the null hypothesis and detect small genetic differences. The hypothesis testing framework implies rejecting the null hypothesis, so it does not work well for detecting the genetic identity, and calculating the power is meaningless. Problems of the null hypothesis testing framework are discussed in Cohen (1994) and Hagen (1997).

An effective method of determining genetic identity is to examine the genetic distances between populations. If an estimated confidence interval of the genetic distance between two populations includes 0, we could conclude that the populations are genetically identical or not statistically significantly different. There are several measures for the genetic distance (Nei 1987). However, the sample distributions of these genetic distances are unknown. It is then inappropriate to estimate the confidence intervals of the genetic distance using asymptotic variances of the estimator.

In this article, we develop a Bayesian estimating procedure to measure genetic distances between populations from allele frequencies. We can directly evaluate the probability distribution of the genetic distance from its posterior distribution. The general method developed

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here is extended to estimate the effective population size termed N_e in a population based on temporal changes in allele frequencies. The joint marginal-likelihood function derived here coincides with the likelihood function to estimate the rate of gene flow into island populations using the sample allele frequency from a number of islands (Rannala and Hartigan 1996).

METHODS

Let the frequencies of k alleles of two populations to be compared be $p_{11}, \cdots, p_{1k}$ and $p_{21}, \cdots, p_{2k}$. Sanghvi (1953) proposed that the genetic distance between two populations be determined by

$$D = \sum_{i=1}^{k} \frac{2(p_{1i} - p_{2i})^2}{p_{1i} + p_{2i}}. \quad (1)$$

We use this distance as a natural measure of the genetic distance between populations, which takes values between 0 and 4. It is known that $2n_1.n_2.\hat{D}/(n_1. + n_2.)$ follows a χ^2 distribution with degree of freedom $k - 1$ when $p_{1i} = p_{2i} = p_i$ for $i = 1, \cdots, k$ (Nei 1987), where $n_1.$ and $n_2.$ are sample sizes (individuals) of the two populations, and $\hat{D}$ is the estimator obtained by substituting sample frequencies in Equation 1. However, the distribution of $\hat{D}$ is unknown when $p_{1i} \neq p_{2i}$ for $i = 1, \cdots, k$. It is then inappropriate to evaluate the confidence interval of D using an asymptotic variance of $\hat{D}$, although it can be derived. Here we directly evaluate the posterior probability density of the genetic distance measure using a Bayesian framework.

Prior and posterior distribution of *D*: It is not easy to describe a reasonable prior distribution of D, especially when we compare more than two populations. Alternatively, we set a prior for allele frequencies. Let the allele frequency of a population be $\boldsymbol{p} = (p_1, \cdots, p_k)'$ and the sample count be $\boldsymbol{n} = (n_1, \cdots, n_k)'$, where $\Sigma p_i = 1$ and $\Sigma n_i = 2n$ (n individuals). When the sample is collected by a simple random sampling procedure with replacement, $\boldsymbol{n}$ follows a multinomial distribution. A β distribution is known as a conjugate prior of the binomial parameter p. A Dirichlet distribution is a conjugate prior of multinomial proportions, which is an extension of a β distribution (Johnson and Kotz 1969; Lee 1989):

$$\pi(\boldsymbol{p}|\boldsymbol{\alpha}) = \frac{\Gamma(\Sigma_{i=1}^{k}\alpha_i)}{\Pi_{i=1}^{k}\Gamma(\alpha_i)} \prod_{i=1}^{k} p_i^{\alpha_i - 1}. \quad (2)$$

Here, $\boldsymbol{\alpha} = (\alpha_1, \cdots, \alpha_k)'$ are regarded as hyperparameters specifying the prior distribution. We use this distribution as a prior for allele frequencies.

The posterior distribution is obtained by multiplying the likelihood function, which is multinomial distribution in this case, by the prior. The posterior distribution of $\boldsymbol{p}$ is then given by

$$P(\boldsymbol{p}|\boldsymbol{n}) \propto \frac{(\Sigma_{i=1}^{k} n_i)!}{\Pi_{i=1}^{k} n_i!} \frac{\Gamma(\Sigma_{i=1}^{k}\alpha_i)}{\Pi_{i=1}^{k}\Gamma(\alpha_i)} \prod_{i=1}^{k} p_i^{\alpha_i + n_i - 1}$$
$$\propto \frac{\Gamma(\Sigma_{i=1}^{k}(\alpha_i + n_i))}{\Pi_{i=1}^{k}\Gamma(\alpha_i + n_i)} \prod_{i=1}^{k} p_i^{\alpha_i + n_i - 1},$$

which is again a Dirichlet distribution with parameters modified by the data $n_i + \alpha_i$ (Lange 1995; Weir 1996). Given $\boldsymbol{\alpha} = (\alpha_1, \cdots, \alpha_k)'$, we can obtain a posterior distribution of $\boldsymbol{p}$ by generating Dirichlet random numbers with parameter $\boldsymbol{\alpha} + \boldsymbol{n}$ using Monte Carlo simulations. Using independent Dirichlet random numbers for posterior distributions of population allelic frequencies, we can obtain a posterior distribution of D using Equation 1. The number of each Monte Carlo simulation is set to 10,000, so 10,000 D are calculated from the 10,000 sets of $\boldsymbol{p}$ between two populations. The posterior probability density function is estimated on the basis of the histogram of D with the number of classes of 100 by using the function "density" of S language version 4 (Chambers and Hastie 1992). For multilocus data, the mean of the genetic distances at J loci is calculated as $D = \Sigma_{j=1}^{J}\hat{D}_j/J$.

Empirical Bayes procedure: The primary disadvantage of using a Bayesian analysis for allele frequency estimation is that there is no obvious way of selecting a reasonable prior (Lange 1995). The Dirichlet distribution with $\alpha_1 = \cdots = \alpha_k = ½$ is a noninformative prior (Box and Tiao 1992). Here we adopt an empirical Bayes procedure to avoid dependence to priors. This procedure estimates the hyperparameters $\boldsymbol{\alpha}$ by maximizing the marginal-likelihood function (Maritz and Lwin 1989),

$$\tilde{L}(\boldsymbol{\alpha}|\boldsymbol{n}) = \int \cdots \int P(\boldsymbol{n}|\boldsymbol{p})\pi(\boldsymbol{p}|\boldsymbol{\alpha})\,d\boldsymbol{p}$$
$$= \frac{(\Sigma_{i=1}^{k} n_i)!}{\prod n_i!} \frac{\Gamma(\Sigma_{i=1}^{k}\alpha_i)}{\Pi_{i=1}^{k}\Gamma(\alpha_i)} \int \cdots \int \prod_{i=1}^{k} p_i^{\alpha_i + n_i - 1}\,d\boldsymbol{p}$$
$$= \frac{(\Sigma_{i=1}^{k} n_i)!}{\prod n_i!} \frac{\Gamma(\Sigma_{i=1}^{k}\alpha_i)}{\Gamma(\Sigma_{i=1}^{k}\alpha_i + \Sigma n_i)} \prod_{i=1}^{k} \frac{\Gamma(\alpha_i + n_i)}{\Gamma(\alpha_i)},$$

which is also given in Lange (1995) and Weir (1996). The distribution is known as a Dirichlet-multinomial distribution (Lange 1995; Weir 1996), which is a generalization of the β-binomial distribution.

Lange (1995) estimated the hyperparameters from single-locus data using Newton's method. Here we estimate them from multilocus data by maximizing Equation 3 using a simplex minimization for the negative logarithm of Equation 3. Assuming that $\boldsymbol{\alpha}$ is the same for H populations (samples) to be compared and $\Sigma\boldsymbol{\alpha}_i$ is also the same for J loci, the joint marginal likelihood is then given by

$$\tilde{L}(\alpha_{1j}, \cdots, \alpha_{kj-1,j} (j = 1, \cdots, J), \sigma^2|\boldsymbol{n})$$
$$= \prod_{j=1}^{J}\prod_{h=1}^{H}\left\{ C_{jh} \frac{\Gamma(\Sigma_{i=1}^{k_j}\alpha_{ij})}{\Gamma(\Sigma_{i=1}^{k_j}\alpha_{ij} + \Sigma_{i=1}^{k_j} n_{hij})} \prod_{i=1}^{k_j} \frac{\Gamma(\alpha_{ij} + n_{hij})}{\Gamma(\alpha_{ij})} \right\}, \quad (3)$$

where $C_{jh} = (\Sigma_{i=1}^{k_j} n_{hij})!/\Pi_{i=1}^{k_j} n_{hij}!$ is a constant term for the combination of the multinomial likelihood that can be excluded from the estimation procedure.

Parameter σ^2 is the dispersion parameter that defines the magnitude of overdispersion; *i.e.*, the variance of the response Y exceeds the nominal variance (McCullagh and Nelder 1983). For example, the expectation of the binomial random variables of a sample size m is $E[Y] = mp$ and the variance is $V[Y] = mp(1 - p)$. If there is overdispersion, the variance is $V[Y] = \sigma^2 mp(1 - p)$ though the expectation remains the same, where Y has a density of a β-binomial distribution. For a multinomial event with overdispersion, the variance-covariance matrix of $\boldsymbol{Y}$ is σ^2 times larger than that of the multinomial distribution, where $\boldsymbol{Y}$ has a density of a Dirichlet-multinomial distribution.

Johnson and Kotz (1969) showed that the variance-covariance matrix of a Dirichlet-multinomial distribution is $(\Sigma_{i=1}^{k} n_i + \Sigma_{i=1}^{k}\alpha_i)/(1 + \Sigma_{i=1}^{k}\alpha_i)$ times larger than that of the multinomial distribution. Hence the relationship between the dispersion parameter and the hyperparameters for a population is given by

$$\sigma^2 = \frac{\Sigma_{i=1}^{k} n_i + \Sigma_{i=1}^{k}\alpha_i}{1 + \Sigma_{i=1}^{k}\alpha_i}. \quad (4)$$

We assume equal overdispersion effects for all loci, so the total of the hyperparameters θ (hereafter we use θ for $\Sigma\alpha_i$) is the same for all loci, which gives the expression for σ^2 as

$$\sigma^2 = \frac{2\bar{n} + \theta}{1 + \theta}. \quad (5)$$

Here $2\bar{n}$ is the mean number of genes of H populations given by $2\bar{n} = \Sigma_{h=1}^{H} 2n_h/H$. Given the estimate of σ^2, we have the estimator for θ as

$$\hat{\theta} = \frac{2\bar{n} - \hat{\sigma}^2}{\hat{\sigma}^2 - 1}. \quad (6)$$

We estimate $\Sigma_{j=1}^{l}(k_j - 1) + 1$ free parameters numerically, including σ^2, which is assumed to be the same among loci, and $\alpha_{1j}, \cdots, \alpha_{k_j-1,j}$ for locus j, and α_{kj} is estimated by $\hat{\theta} - \Sigma_{i=1}^{k_j-1}\hat{\alpha}_{ij}$.

The binomial and multinomial counts are assumed to be taken by a simple random sampling, so the dispersion parameter σ^2 is considered to indicate the magnitude of overshooting from a simple random sample. McCullagh and Nelder (1983) stated that "The simplest and perhaps the most common mechanism of overdispersion is clustering in the populations." Kitada *et al.* (1994) estimated the dispersion parameter for fish tag recovery data and showed that the variances of the estimated mortality rates were $\sim\hat{\sigma}^2(= 14.73)$ times larger than those assuming the multinomial model, which was considered to be caused by the aggregation of the tagged fish in the fishing ground. In genetic data analysis, overdispersion corresponds to the variance of an allele frequency exceeding the nominal variance of a simple random sample from a gamete pool. If there are subpopulations divided spatially in a survey area, a sample allele frequency from the area might be overdispersed even if a simple random sampling is performed. If a cluster of a genotype is taken, a sample allele frequency from a population might be also overdispersed. One can then estimate overdispersion based on several sets of allele frequencies obtained from the survey area.

Standardized genetic distance: When allele frequencies at J loci are obtained from genetically identical populations, $2n_1.n_2.\Sigma_{j=1}^{l}\hat{D}_j/(n_1. + n_2.)$ follows a χ^2 distribution with a degree of freedom of $\Sigma(k_j - 1)$ asymptotically (Nei 1987). The shape of the distribution varies with the sample sizes and degree of freedom. When larger numbers of individuals are sampled, the distribution is farther from 0 even if the genetic difference is small. Suppose the case for $n_1. = n_2. = n.$, the above statistics become $n.\Sigma_{j=1}^{l}\hat{D}_j$ and take a value proportional to the sample size. It is then not convenient to make D an index of the genetic distance.

Here we standardize D and propose a general index for the genetic distance. Performing a square root transformation to make the variance independent of the mean (Snedecor and Cochran 1967) and subtracting the expected value of I (the derivation is given in the appendix), we obtain a standardized genetic distance as

$$I = \sqrt{\frac{2n_1.n_2.\Sigma_{j=1}^{l}\hat{D}_j}{(n_1. + n_2.)\sigma^2}} - \sqrt{2}\frac{\Gamma((\Sigma_{j=1}^{l}(k_j - 1) + 1)/2)}{\Gamma(\Sigma_{j=1}^{l}(k_j - 1)/2)}, \quad (7)$$

which follows a normal distribution with mean 0 and variance 0.5 under the condition of $\boldsymbol{p}_1 = \boldsymbol{p}_2$.

The χ^2 distribution of $2n_1.n_2.\Sigma_{j=1}^{l}\hat{D}_j/(n_1. + n_2.)$ assumes that $2n_1$ and $2n_2$ genes are taken by a binomial sampling from a population. For this case, σ^2 in the first term of Equation 7 equals 1. However, if there is overdispersion, σ^2 becomes active and takes a value larger than 1. If the overdispersion is neglected, the genetic distance is then overestimated and the scale of the distribution of $2n_1.n_2.\Sigma_{j=1}^{l}\hat{D}_j/(n_1. + n_2.)$ becomes σ^2 times larger than that under the previously stated assumption. The dispersion parameter σ^2 corrects this effect.

Effective population size: The effective population size is estimated from the temporal variation of allele frequencies in a population. Since the observed variance of the allele frequencies includes the sampling variance in addition to the genetic drift, we subtract the sampling variance when estimating the effective population size. Let us assume that we have two samples with sizes n_0 and n_t from the population at generations 0 and t, respectively. The empirical Bayes procedure developed here can be extended to obtain the posterior distributions of the effective population size N_e by using the posterior distribution of F-statistics calculated from the posterior distribution of allele frequencies.

The standardized variance of allele frequency change measured by *F*-statistics has been used to estimate N_e (Krimbas and Tsakas 1971; Nei and Tajima 1981; Pollak 1983; Waples 1989). Among *F*-statistics, F_k proposed by Pollak (1983) is similar in form to *D* and is given by

$$F_k = \frac{1}{k-1}\sum_{i=1}^{k}\frac{2(p_{0i}-p_{ti})^2}{p_{0i}+p_{ti}}. \quad (8)$$

For the case of multiple loci, F_k is calculated by $F_k = \Sigma_j(k_j - 1)F_{kj}/\Sigma_j(k_j - 1)$ from Nei and Tajima (1981), where F_{kj} is F_k at the *j*th locus. Without overdispersion, N_e is estimated by

$$\hat{N}_e = \frac{t}{2[\hat{F}_k - 1/(2n_0) - 1/(2n_t) + 1/N]} \quad (9)$$

for plan I, where the sample is taken after reproduction. For plan II, where the sample is taken before reproduction, the term $1/N$ is eliminated, where $\hat{F}_k$ is the estimator obtained by substituting sample frequencies in Equation 8 and *N* is the census size for a population (Waples 1989, Equations 11 and 12).

Equation 9 assumes that $2n_0$ and $2n_t$ genes are taken by a binomial sampling from the population. If there is overdispersion, F_k is overestimated, which leads to underestimation of N_e. Since the effective sample size is obtained by discounting the apparent sample size by dispersion parameters, Equation 9 is modified as

$$\hat{N}_e = \frac{t}{2[\hat{F}_k - \hat{\sigma}^2/(2n_0) - \hat{\sigma}^2/(2n_t) + 1/N]}. \quad (10)$$

CASE STUDIES

Red sea bream: To evaluate genetic distances, we first analyzed the data of four populations of red sea bream (*Pagrus major*) from Tabata and Mizuta (1997; Table 1). From the fragment pattern of mtDNA D-loop regions with six restriction enzymes, 48 haplotypes were obtained for four wild populations. We decomposed the haplotype frequency to six allelic frequencies for each restriction enzyme and eliminated *Hae*III and *Msp*, which showed little or no polymorphism, from the analysis.

The estimate of the total hyperparameters was 106.553 for each locus (Table 1), and the dispersion parameter was estimated at 1.80 by maximizing Equation 3. Here $2\bar{n}_\cdot = (72 + 95 + 93 + 90)/4 = 87.5$ because mtDNA is a haploid. With a prior distribution specified by these parameters, we obtained the posterior distribution of *D* by dividing ΣD_j over four loci by the number of loci (Table 2). As an example, the histogram and estimated density function of the posterior distribution of *D* at *Hin*fI between Tanabe Bay and Tomogashima Channel is shown in Figure 1. D_{12} in Figure 2 was obtained as the mean of such four posterior genetic distances at the four loci. It should be noted that the posterior distances were overestimated including the overdispersion and the posterior distributions in Figure 2 were then overestimated. The means and SDs of D_{12}, D_{13}, and D_{14} were about two times larger than D_{23}, D_{24}, and D_{34}; however, they might include the effect of the smaller sample size of population 1 (Tanabe Bay).

The posterior distribution of the standardized genetic distance took the overdispersion and sample size difference into account. The means of I_{12}, I_{13}, and I_{14} ranged from 0.2706 to 0.4671, whereas those of I_{23}, I_{24}, and I_{34} took negative values. The SDs ranged from 0.53 to 0.58 and took almost the same values. The genetic differences with population 1 (I_{12}, I_{13}, and I_{14}) looked larger than the others (Table 3). However, the posterior distributions of *I* overlapped well with the theoretical distribution of no genetic difference (Figure 3).

We estimated the 95% confidence interval of the dispersion parameter to be from 1.72 to 1.88 by the likelihood-ratio test. The lower limit of the dispersion parameter corresponds to the upper limit of the genetic distance, from which we evaluate the difference. The means of the posterior distributions for the lower limit of the dispersion parameter were increased from 8 to 27% and SDs remained the same (Table 3), but the posterior distributions of *I* were almost the same as those for the point estimate of the overdispersion and still overlapped well with the theoretical distribution (Figure 3).

The value of 95% upper limit of the credibility region of the theoretical normal distribution of *I* with mean of 0 and variance of 0.5 is 1.16. All posterior means were <1.16, and the credibility regions included 0; hence we concluded that there was no genetic difference between the four populations of red sea bream. This finding agreed with the result of the original authors, who reported that the Roff test did not reject the homogeneity of the haplotype frequencies (Tabata and Mizuta 1997, $p = 0.219$). Nevertheless, they rejected the hypothesis by the pairwise comparison. From the results of our test, however, we argue that it was inappropriate analysis.

Herring: Stock enhancement of herring (*Clupea pallasii*) has been performed in Akkeshi Bay, Hokkaido (Japan). Because the matured herrings migrate to Akkeshi Bay to spawn, they are considered to have originated from Lake Akkeshi and Akkeshi Bay. Although wild adult fish that migrated to the bay are used for artificial spawning to produce juveniles every year, it still may be important to monitor the genetic change and estimate the effective population size to maintain the wild stock.

Temporal changes in allozyme allele frequencies were obtained by combining two studies on the same loci by Ando and Ohkubo (1997) and Hotta *et al.* (1999; Table 4). Independent samples were taken in March and April 1993. In 1996, males and females were taken separately from the sample, hence they were not inde-

TABLE 1

Allele frequencies of the mtDNA D-loop region from Tabata and Mizuta (1997) and estimated hyperparameters for four populations of red sea bream from eastern Japan

Sample size:	Tanabe Bay 72	Tomogashima Channel 95	Sea of Japan 93	Bingo Nada 90	Hyperparameter
*Hin*fI	0.458	0.411	0.376	0.378	43.855
	0.375	0.442	0.409	0.456	43.872
	0.167	0.147	0.215	0.166	18.826
*Msp*I	0.903	0.863	0.882	0.867	92.864
	0.097	0.137	0.118	0.133	13.688
*Taq*I	0.958	0.811	0.806	0.856	91.248
	0.042	0.189	0.194	0.144	15.305
*Rsa*I	0.097	0.137	0.215	0.178	17.015
	0.264	0.305	0.312	0.266	30.253
	0.306	0.316	0.312	0.289	32.871
	0.180	0.116	0.075	0.078	11.802
	0.153	0.126	0.086	0.189	14.613

pendent. For the purposes of our case study, we treated the data as if they were taken independently.

The estimate of the total hyperparameters was 130.956 for each locus (Table 4), and the dispersion parameter was estimated at 2.39, with $2\bar{n}_{\cdot} = (206 + 214 + 168 + 148)/4 = 184$. The posterior distribution of F_k estimate was calculated from each of two sets of posterior distributions of allele frequencies for 1993 and 1996 by

$$F_k = \frac{1}{4}\sum \frac{\Sigma_{j=1}^{J}(k_j - 1)\hat{F}_{kj}}{\Sigma_{j=1}^{J}(k_j - 1)}. \qquad (11)$$

F_k ranged from 0.0014 to 0.0814 with the mean and SD of 0.0226 and 0.0105, respectively. The posterior distribution of F_k is shown in the left side of Figure 4.

Most of the matured herring migrating to Akkeshi Bay to spawn are in their second year of life; the remainder are in their third year. The age composition of the spawners was surveyed and estimated at 0.9 and 0.1 for each age class by the Japan Sea-Farming Association. The expected number of generations can be used for t in the estimating equations of N_e because the expectation of the F-statistics was approximated to be linear with t as shown in Waples (1989, p. 382). We estimated the expected number of generations at 1.48 (= 3/2.1) divided by the number of years between samples by the mean age of spawners as Miller and Kapuscinski (1997) did. The samples were taken before reproduction, so Equation 10 was used by eliminating the term of $1/N$ and substituting $(206 + 214)/2 = 210$ for $2n_0$ and $(168 + 148)/2 = 158$ for $2n_t$. The posterior means of N_e estimates and 95% credibility region of N_e are given in Table 5.

The dispersion parameter was estimated at 2.39 with a 95% confidence interval from 1.00 to 7.51. From a conservation viewpoint, it is better to consider the lower limit of N_e. The lower limit of the dispersion parameter evaluates the upper limit of F_k corresponding to the lower limit of N_e. The lower limit of σ^2 was 1.00. Corresponding with that, no overdispersion arose and no subpopulation existed. The number of simulations with a negative value of N_e estimate was 1221 in 10,000 trials. When $F_k \leq [1/(2n_0) - 1/(2n_t)]$, the only feasible estimate of N_e is infinity (Waples 1989). The mean of the positive N_e estimate was 350, and the 95% credibility region was estimated from 20 to infinity (Table 5). The posterior distribution of the positive N_e estimate is shown in the right side of Figure 4; it suggests the order of the effective population size of herring, even though the upper limit was not estimated.

Northern pike: We analyzed the data from Miller and Kapuscinski (1997) for comparison. Temporal changes in microsatellite allele frequency at seven loci were typed in the northern pike (*Esox lucius*) population of Lake Escanaba, Wisconsin. Of the seven loci, five had

TABLE 2

Means and SDs of the posterior distribution of the genetic distance for the red sea bream populations

	D_{12}	D_{13}	D_{14}	D_{23}	D_{24}	D_{34}
Mean	0.0585	0.0523	0.0530	0.0229	0.0241	0.0244
SD	0.0207	0.0196	0.0192	0.0112	0.0116	0.0118

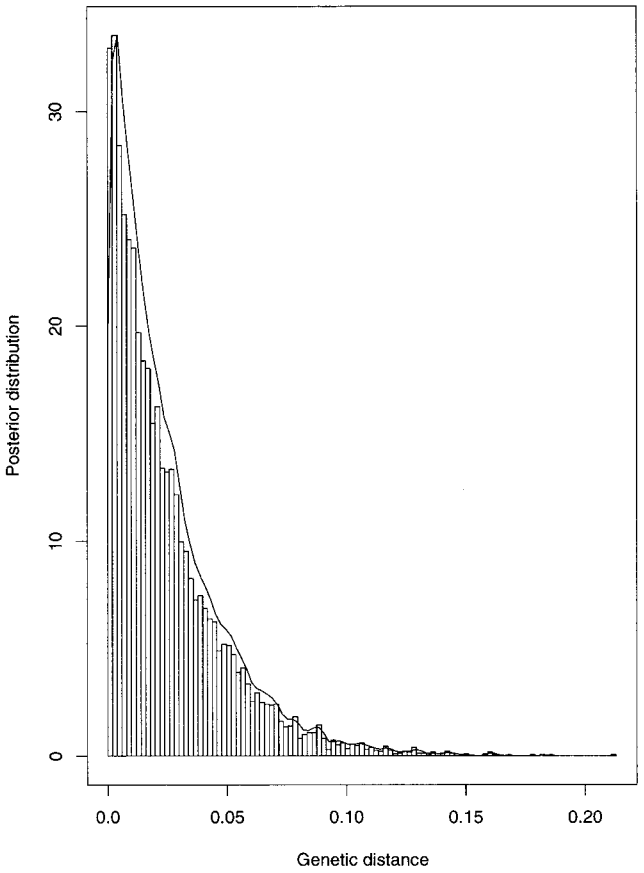

FIGURE 1.—A histogram and the estimated density function of the posterior distribution of the genetic distance between red sea bream populations of Tanabe Bay and Tomogashima Channel for *Hin*fI using data given in Table 1. D_{12} in Figure 2 was obtained as the mean of the four posterior genetic distances for the four loci.

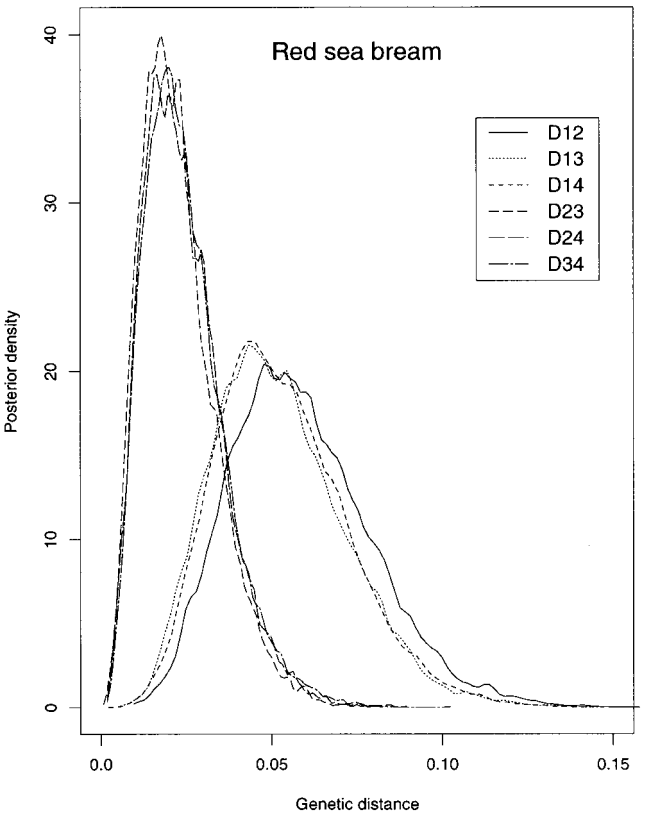

FIGURE 2.—Posterior distributions of the genetic distance (D) between four populations of red sea bream using data given in Table 1.

two alleles and two had three alleles. Following the data processing techniques of WILLIAMSON and SLATKIN (1999), we also combined the two least common allelic classes for the two loci with three alleles and created a diallelic frequency set. To estimate the hyperparameters, we allocated sample sizes of 86 for 1961, 110 for 1977, and 72 for 1993 according to the diallelic frequencies for each locus to obtain the number of individuals corresponding to the frequencies. Because the data had one sample for each year, it was not possible to estimate the hyperparameters for each locus. Therefore, we assumed that the seven loci were independent of each other and had common hyperparameters. The two common hyperparameters and the dispersion parameter were estimated at 16.232, 3.089, and 9.74, with $2\bar{n}. = (172 + 220 + 144)/3 = 178.7$.

F_k between the year of 1977 and 1993 ranged from 0.0087 to 0.1264 with the mean and SD being 0.0479 and 0.0150, respectively. The posterior distribution of F_k is given in the left side of Figure 5. The posterior distribution of the N_e estimate was obtained by substituting the posterior distribution of F_k into Equation 10, eliminating the term $1/N$, with $t = 4$, as given in MILLER and KAPUSCINSKI (1997). The mean of the positive N_e estimate was 73 and a 95% credibility region neglecting the overdispersion was estimated from 29 to 190 (Table 6). The number of negative N_e estimates was 6 in 10,000 trials. MILLER and KAPUSCINSKI's estimates for 1977 and 1993 data were 0.038 for F_k and 72 for N_e with a 95% confidence interval from 17 to 258. Our posterior mean of F_k was 1.26 times larger and that of the N_e estimate coincided with a 67% narrower confidence interval (Table 6).

The 95% confidence interval of the dispersion parameter was estimated from 5.51 to 18.80. For the 95% lower limit of the dispersion parameter, the number of simulations with a negative value of N_e estimate was 8480 in 10,000 trials. The mean of the positive N_e estimates was 1065 and the 95% credibility region was estimated from 123 to infinity (Table 6). The posterior distribution of the positive N_e estimate, neglecting the overdispersion ($\sigma^2 = 1$), and for the lower limit of $\hat{\sigma}^2 (= 5.51)$ are given in the right side of Figure 5. In this example, we can see the effect of the overdispersion on the posterior distribution of N_e estimate. The estimate of N_e, neglecting the overdispersion, agreed well with the estimate of MILLER and KAPUSCINSKI (1997).

Ayu broodstock: Ayu (*Plecoglossus altivelis*) is the most popular target species of recreational anglers in rivers and streams in Japan. A total of 300 million juveniles

TABLE 3

Means and 95% credibility regions of the posterior distribution of the standardized genetic distance for the red sea bream populations

	$\sigma^2 = 1.80^a$			$\sigma^2 = 1.72^b$		
	Mean	SD	95% CR	Mean	SD	95% CR
I_{12}	0.4671	0.5774	[−0.4819, 1.4232]	0.5448	0.5914	[−0.4272, 1.5240]
I_{13}	0.2706	0.5747	[−0.6706, 1.2169]	0.3435	0.5886	[−0.6205, 1.3127]
I_{14}	0.2735	0.5567	[−0.6259, 1.1977]	0.3464	0.5702	[−0.5747, 1.2931]
I_{23}	−0.6241	0.5284	[−1.4582, 0.2767]	−0.5729	0.5411	[−1.4272, 0.3497]
I_{24}	−0.5866	0.5332	[−1.4226, 0.3163]	−0.5345	0.5461	[−1.3907, 0.3903]
I_{34}	−0.5768	0.5276	[−1.4047, 0.3319]	−0.5244	0.5404	[−1.3723, 0.4062]

CR, credibility region.
[a] Point estimate of the dispersion parameter.
[b] 95% lower limit of the dispersion parameter.

are released every year, of which hatchery-produced fish comprise ~30%. The life span of ayu is 1 year. They spawn in a river from September to November and die after spawning. Hatched larvae go down to the sea and winter there. The upstream run of wild ayu juveniles begins from the coast in late March to early April and is over by early July. Soon after, they mature, spawn from September to November, and then die after spawning.

Hatcheries have commonly cultured broodstocks over generations. At the Gunma Prefecture Fisheries Experimental Station, adult ayu have been cultured over 27 generations. About 3000–4000 fish have been reared every year as broodstock, some of which are used for artificial fertilization. In 1996, ~850 females and 650 males were used. The temporal changes in allozyme allele frequencies of the ayu broodstock were reported by Yoshizawa (1997; Table 7). These samples in Table 7 were taken after artificial fertilization from two rearing tanks in which males and females were kept separately.

The total of the hyperparameters was estimated at 28.576 for each locus (Table 7), and the dispersion parameter was estimated at 5.86, with $2\bar{n}. = (190 + 210 + 128 + 130 + 100 + 100)/6 = 143$. The 95% confidence interval of the dispersion parameter was estimated to range from 3.04 to 13.49. We calculated four F_k's on the basis of temporal changes in allele frequen-

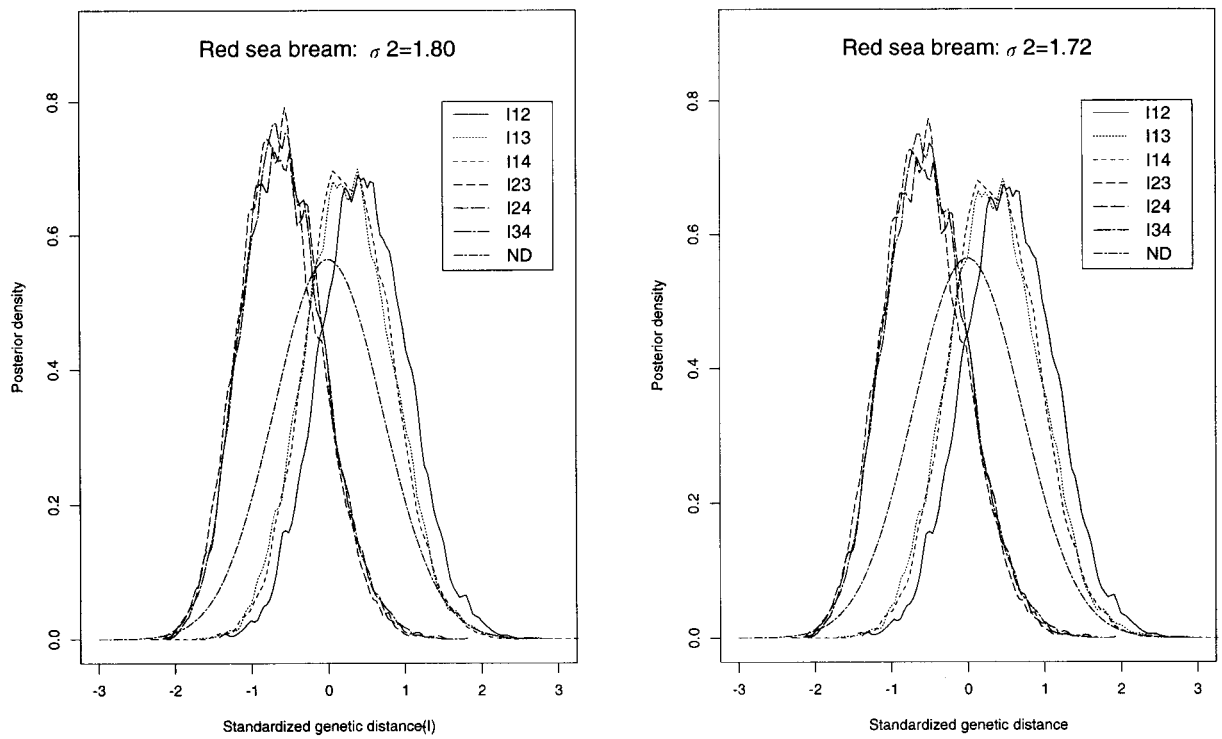

Figure 3.—Posterior distributions of the standardized genetic distance (I) for the red sea bream populations taking the point estimate of the dispersion parameter 1.80 (left) and the 95% lower limit 1.72 (right) into account.

TABLE 4

Temporal changes in allozyme allele frequencies and estimated hyperparameters of herring in Akkeshi Bay

	1993[a]		1996[b]		
	March	April	Male	Female	
Sample size:	103	107	84	74	Hyperparameter
Gpi	0.282	0.243	0.191	0.149	28.630
	0.718	0.757	0.809	0.851	102.326
Pgm	0.447	0.393	0.321	0.324	48.993
	0.553	0.607	0.679	0.676	81.963

[a] Ando and Ohkubo (1997).
[b] Hotta *et al.* (1999).

cies observed in the first (1996–1997; F_1) and second (1997–1998) time intervals (F_2), over the entire interval (1996–1998; F_3), and for the entire interval based on the pooled F for the first two intervals, as Miller and Kapuscinski (1997) did. For the last case, Miller and Kapuscinski (1997) used the sum of F_1 and F_2 for the entire interval, but we used the mean for the two intervals (F_{mean}), which gives the same form of N_e estimate by Equation 10, substituting n_0 by the harmonic mean of the sample size of the first and second year, and n_t by that of the second and third year. When t is equal for the two intervals, the estimate of N_e derived from pooled F_k is the harmonic mean for both sampling periods (Nei and Tajima 1981; Pollak 1983; Waples 1989; Miller and Kapuscinski 1997; Williamson and Slatkin 1999).

The values of F_k calculated by Equation 11 were varied using four combinations for two samples in each sampling year, reflecting the variation in allele frequencies for each sampling period. The posterior mean and SD of F_3 were the largest, and the SD of F_{mean} was the smallest but almost the same as that of F_1 (Table 8). The posterior distributions of F_k are illustrated in the left side of Figure 6.

We fixed the generation time at $t = 1.0$ for the first and second time intervals and had $t = 2.0$ for the entire interval because the life span of ayu is 1 year. The samples were taken after reproduction, so we used Equation

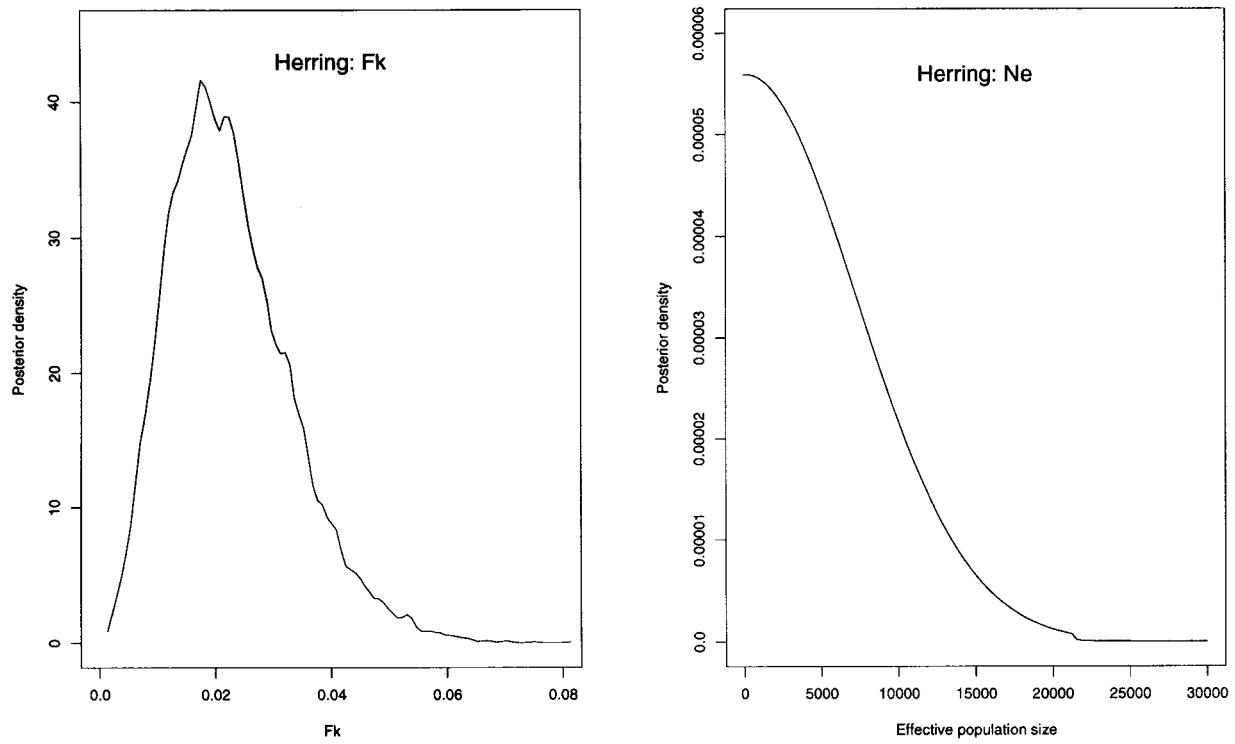

Figure 4.—Posterior distributions of the standardized variance of allele frequency change F_k and effective population size N_e of herring for the 95% lower limit of the dispersion parameter ($\sigma^2 = 1$) using data in Table 4.

TABLE 5

Means and 95% credibility regions of the posterior distribution of the effective population size of herring in Akkeshi Bay obtained using data in Table 4

σ^2	Mean[a]	95% CR	∞/10,000[b]
1.00[c]	350	[20–∞]	1,221
2.39[d]	16,841	[35–∞]	6,832
7.51[e]	∞	[∞–∞]	10,000

[a] Mean of positive N_e.
[b] Number of N_e that took ∞ in 10,000 simulations.
[c] 95% lower limit of the dispersion parameter (no overdispersion).
[d] Point estimate of the dispersion parameter.
[e] 95% upper limit of the dispersion parameter.

TABLE 6

Means and 95% credibility regions of the posterior distribution of the effective population size of northern pike obtained using 1977–1993 data from Miller and Kapuscinski (1997)

σ^2	Mean[a]	95% CR	∞/10,000[b]
1.00[c]	73	[29–190]	6
M and K[d]	72	[17–258]	
5.51[e]	1,065	[123–∞]	8,480
9.74[f]	20,606	[∞–∞]	9,998

[a] Mean of positive N_e.
[b] Number of N_e that took ∞ in 10,000 simulations.
[c] No overdispersion is assumed.
[d] Estimated by Miller and Kapuscinski (1997).
[e] 95% lower limit of the dispersion parameter.
[f] Point estimate of the dispersion parameter.

10, substituting $2n_0 = (190 + 210)/2 = 200$, $2n_t = (100 + 100)/2 = 100$, and $N = 1500$, which was the total number of individuals used for artificial fertilization.

We made four estimates of N_e on the basis of F_1, F_2, F_3, and F_{mean}. Estimates for the 95% lower limit of the dispersion parameter (= 3.04) are given in Table 8. The posterior mean obtained using F_3 was the largest with the largest SD, and that for F_{mean} was second with a smaller SD. The N_e estimate that took ∞ in 10,000 simulations was 8677 when using F_{mean}, and that for F_3 was 4927. This is because the sampling correction term in the denominator of Equation 10 took the very similar values of 0.0912 for F_3 and 0.0928 for F_{mean}, despite the posterior mean of F_{mean} being smaller than that of F_3, as shown in the left side of Figure 6. The posterior distributions of the positive N_e estimate obtained by using F_1, F_2, F_3, and F_{mean} for the lower limit of $\hat{\sigma}^2$ are given in the right side of Figure 6, showing a larger variance of the estimate based on F_3 than those derived from F_1, F_2, and F_{mean}.

We failed to estimate the upper limit of the credibility regions because of large sampling variances with overdispersion. However, when the numbers of breeding males N_m and females N_f are given, which is difficult to

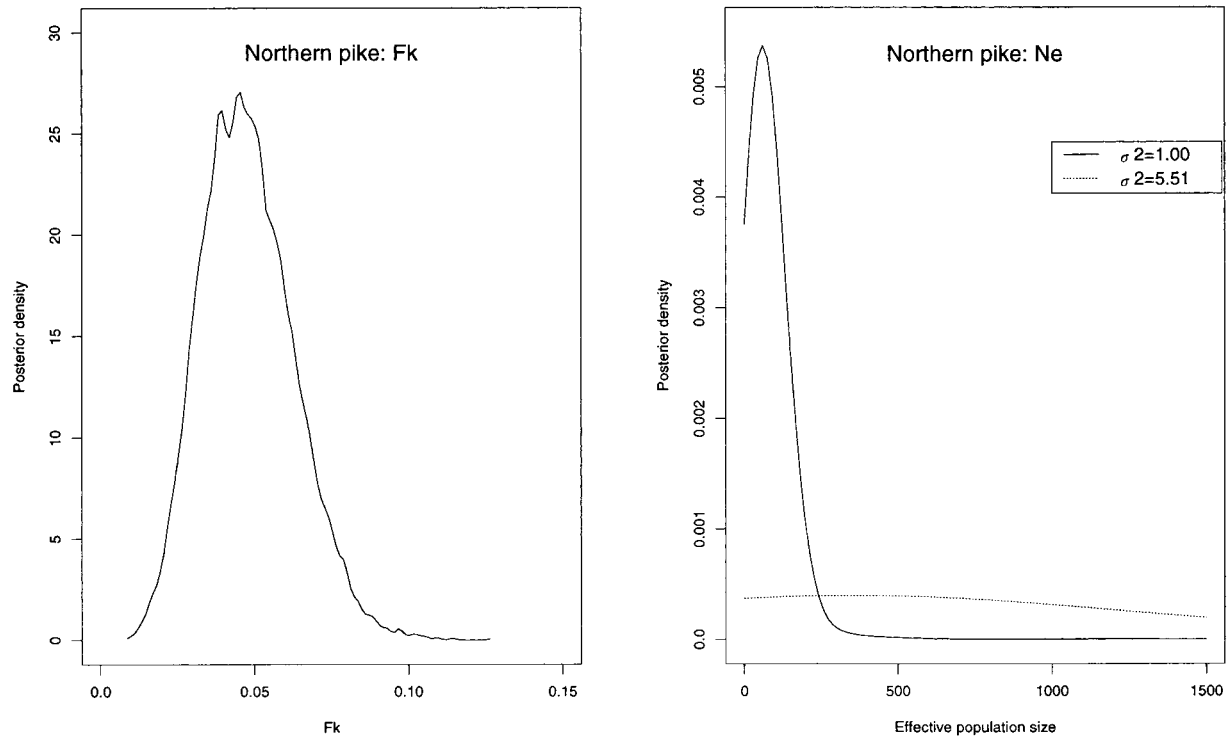

Figure 5.—Posterior distributions of the standardized variance of allele frequency change F_k and effective population size N_e of northern pike for the 95% lower limit of the dispersion parameter ($\sigma^2 = 5.51$) and that with no overdispersion ($\sigma^2 = 1.00$) using 1977–1993 data from Miller and Kapuscinski (1997).

TABLE 7

Temporal changes in allozyme allele frequencies from YOSHIZAWA (1997) and estimated hyperparameters of ayu broodstock cultured over 27 generations in Gunma Prefecture

	1996		1997		1997		
Sample size:	Male 95	Female 105	Male 64	Female 65	Male 50	Female 50	Hyperparameter
*Gpi*1	0.184	0.200	0.141	0.192	0.290	0.230	6.341
	0.816	0.800	0.859	0.808	0.710	0.770	22.235
*Mpi*1	0.521	0.400	0.422	0.315	0.280	0.300	10.794
	0.479	0.600	0.578	0.685	0.720	0.700	17.783

know in a wild population but possible in hatcheries, the effective population size is obtained by $N_e = 4N_mN_f/(N_m + N_f)$ (WRIGHT 1931). We obtained a value for N_e of 1473 by using the equation $(4 \times 850 \times 650/(850 + 650))$, which referred to the effective population size where a random mating was performed by artificial fertilization. In a spawning season of ayu, males and females eligible for spawning were selected every day from the broodstock and used for artificial fertilization. The number of females used in an artificial fertilization ranged from ~10 to 20, and the ratio of males to females was ~0.8. The eggs were squeezed from the females and stocked in a stainless bowl and then fertilized by squeezing sperm from individual males. This method of fertilization might not guarantee a random mating of the males and females used; hence, 1473 should be used as the upper limit of the credibility regions instead of ∞ (Table 8). If we neglect overdispersion, the 90% credibility region could be obtained at [13–589] with the posterior mean of 136, which was underestimated.

DISCUSSION

The empirical Bayes procedure developed here makes it possible to describe the skewness of the genetic distance and evaluate genetic differentiation between populations while taking full account of the uncertainty of the sample allele frequencies. When we compare populations in which the genetic differentiation is small, the hypothesis-testing framework cannot accept the null hypothesis of no genetic differentiation in almost all cases, because of the poor statistical power with relatively small sample sizes. The empirical Bayes procedure is effective even in such cases. So we believe it could play an important role in the field of conservation.

This general method can easily be extended to any parameter that is a function of multinomial frequencies. When the parameter of interest is a function of allele frequencies, the posterior distribution of that parameter can be obtained through the function by using the posterior distribution of the allele frequencies, instead of assuming a prior distribution for the parameter.

Overdispersion and empirical Bayes: Until now, models based on a simple random sampling from the gamete pool have been assumed when evaluating allele frequencies. However, as shown in the four case studies treated in this article, a simple random sampling is not necessarily guaranteed. If there are subpopulations divided spatially in a survey area, or a cluster of a genotype is taken, a sample allele frequency might be overdispersed. If overdispersion arises, a sampling variance becomes σ^2 times larger than that for a simple random sampling.

TABLE 8

Means and 95% credibility regions of the posterior distribution of the effective population size of ayu for 95% lower limit of the dispersion parameter (3.04) obtained using data in Table 7

		F_k		N_e			
Period	t[a]	Mean[b]	SD	Mean	SD	95% CR	∞/10,000[c]
1996–1997 (F_1)	1	0.0263	0.0121	350	4,024	[32–∞]	8,453
1997–1998 (F_2)	1	0.0379	0.0189	240	1,388	[17–∞]	8,094
1997–1999 (F_3)	2	0.0474	0.0191	796	18,538	[22–∞]	4,927
1997–1999 (F_{mean})	1	0.0321	0.0120	491	3,294	[35–∞]	8,677
1997–1999 (F_{mean})[d]				136	3,720	[13–589[e]]	377

[a] Number of generations.
[b] Mean of positive N_e.
[c] Number of N_e that took ∞ in 10,000 simulations.
[d] No overdispersion is assumed ($\sigma^2 = 1$).
[e] 90% credibility region.

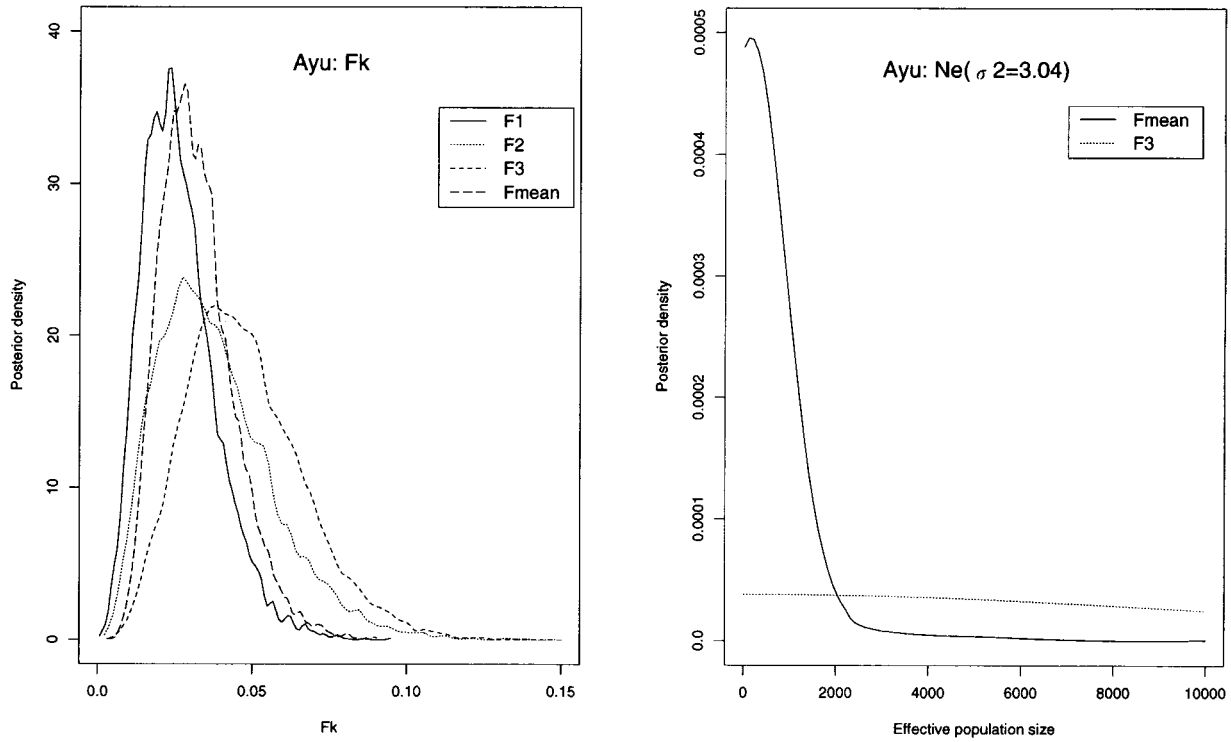

FIGURE 6.—Posterior distributions for the four estimators of the standardized variance of allele frequency change F_k and effective population size N_e of ayu broodstock using 1996–1998 data in Table 7.

This can seriously affect the precision of the estimate of genetic distance and the effective population size. As a result, the genetic distance and *F*-statistics can be overestimated, and the effective population size can be underestimated, if overdispersion is not taken into account in the analysis. Therefore, it is quite important to take overdispersion into account when estimating genetic distance and effective population size.

Sample sizes: If we use the noninformative prior of the Dirichlet distribution (BOX and TIAO 1992), the dispersion parameter might be overestimated for most cases. For example, the dispersion parameter of herring was estimated at 92.5 from the noninformative prior, which was estimated at 2.39 from the empirical Bayes procedure, illustrating the importance of estimating the hyperparameters from the data.

We examined the relationship between sample size and the estimates of the hyperparameters using the data of red sea bream given in Table 1. We estimated the hyperparameters with multipliers of 0.5, 2, 3, and 4 to test each population with the same sample allele frequencies. The estimates of the hyperparameters were stable and not dependent upon sample sizes. This confirmed the robustness of the empirical Bayes procedure (Table 9). However, the dispersion parameter became larger as sample size increased. This is to be expected from the relationship between the total of the hyperparameters, the sample sizes, and the dispersion parameter given by Equation 5.

Suppose there are several subpopulations and the population allele frequencies are largely varied spatially. If the sample sizes are small, one might consider that the large variation in the sample allele frequencies is a function of the small sample size. On the other hand, if the same allele frequencies are obtained for larger sample sizes, one could consider that the large variation comes from the subpopulation structure with confidence. The more samples one draws, the more precisely one can estimate the dispersion parameter. In addition, increasing the number of polymorphic loci to be surveyed may also increase the information available for estimating the dispersion parameter, *e.g.*, the precision of the dispersion parameter estimate in the case of red sea bream, in which the narrowest confidence interval was obtained among our four case studies.

It is also quite important to consider sampling strategies to minimize overdispersion caused by sampling procedures. For example, sampling from different sites and times may be useful to avoid sampling clusters of individuals having the same genotype. Such multiple samples contribute simultaneously to a more precise estimation of the dispersion parameter.

Standardized genetic distance: As I follows the normal distribution, it takes values between $-\infty$ and $+\infty$. For simplicity, let $X = 2n_1.n_2.\Sigma_{j=1}^{l}\hat{D}_j/((n_1. + n_2.)\sigma^2)$ and define the expected value by $E[\sqrt{X}]$. If $\sqrt{X} > E[\sqrt{X}]$, I takes a positive value, and 0 if $\sqrt{X} = E[\sqrt{X}]$. If $\sqrt{X} < E[\sqrt{X}]$, I takes a negative value. As X follows the χ^2 distribution asymptotically when there is no genetic difference, $E[\sqrt{X}]$ is almost equal to the square root of the degrees of freedom of X, which is the number of loci examined. When $\Sigma_{j=1}^{l}\hat{D}_j$ does not increase compared to the increased num-

TABLE 9

Estimated hyperparameters and the dispersion parameter for four populations of red sea bream for sample sizes of 0.5, 2, 3, and 4 times larger than the original one with the same sample allele frequencies

Sample size:	×0.5	Original	×2	×3	×4
θ	106.844	106.553	106.168	106.119	106.075
σ^2	1.40	1.80	2.62	3.44	4.25
*Hin*fI	43.956	43.855	43.603	44.301	43.683
	43.989	43.872	43.887	43.932	43.986
	18.899	18.826	18.679	17.886	18.406
*Msp*I	93.049	92.864	93.007	92.027	93.033
	13.795	13.688	13.161	14.093	13.042
*Taq*I	90.981	91.248	90.853	91.949	91.253
	15.863	15.305	15.315	14.170	14.822
*Rsa*I	16.961	17.015	16.759	16.886	16.383
	30.155	30.253	30.091	29.762	30.310
	32.976	32.871	33.032	32.855	32.791
	11.841	11.802	11.777	11.732	11.910
	14.910	14.613	14.509	14.885	14.682

ber of loci examined, the likelihood for I taking a negative value increases. On the other hand, when $\Sigma_{j=1}^{J}\hat{D}_j$ increases to the increased number of loci examined, the likelihood for I taking a positive value increases. This point illustrates the effectiveness of increasing the number of loci to obtain increased information on the genetic differentiation from the value of the posterior mean of I. Conversely, a negative posterior mean indicates that little information on genetic differentiation will be obtained even if the number of loci is increased, as a function of the small genetic differentiation. This is considered to be the cause of the negative values of the posterior mean for I_{23}, I_{24}, and I_{34}.

Overdispersion and gene flow: Weir (1996) stated that for populations that have reached an equilibrium under the joint effects of drift and mutation or migration, Wright (1945) found that allele frequencies for loci with two alleles had a β distribution, and for multiallele loci the distribution was Dirichlet (Wright 1951). We assumed that the hyperparameters for the β or Dirichlet distributions were common for every sample and locus that was in an overdispersed population. Our assumption corresponds with Wright's (1945, 1951) theories. So if the random sampling is performed, the estimated hyperparameters and dispersion parameter both describe a kind of genetic differentiation between populations that have reached an equilibrium. If all populations mate randomly, the total variance of allele frequency p with two alleles of $2n$ genes is given by Weir (1996)

$$V(\hat{p}) = \frac{p(1-p)}{2n}\{F_{ST}(2n-1)+1\}, \qquad (12)$$

where F_{ST} is the coancestry coefficient of Wright (1951). The second term of Equation 12 corresponds to the dispersion parameter, yielding the relationship

$$\sigma^2 = F_{ST}(2n-1)+1, \qquad (13)$$

from which we can see larger F_{ST} gives larger overdispersion. From Equation 13, we also have the relationship

$$F_{ST} = \frac{\sigma^2-1}{2n-1}. \qquad (14)$$

Rannala and Hartigan (1996) proposed the pseudo-maximum-likelihood method (PMLE) for estimating the rate of gene flow into island populations using the distribution of alleles in samples from a number of islands. We confirmed that their likelihood function for multiple loci (p. 149 Equation 10) coincides with Equation 3 by using the relationship of $\Gamma(n) = (n-1)!$. In PMLE, α_i is treated by θp_i. Here, p_i is a

TABLE 10

Estimated hyperparameters and the dispersion parameter from the mtDNA haplotype distribution among islands for Channel Island foxes (Table 2 of Rannala and Hartigan 1996), using the full-likelihood function (Equation 3)

Parameter	Rannala and Hartigan (PMLE)	This article (MLE)
θ	0.41	0.45
	(±0.35)[a]	[0.18, 0.84][b]
α_1	0.1189	0.0945
α_2	0.0574	0.0428
α_3	0.0082	0.0382
α_4	0.0779	0.0992
α_5	0.1476	0.1760
σ^2	18.38[c]	17.89

[a] SD.
[b] 95% confidence interval estimated by the likelihood-ratio test.
[c] Estimated by Equation 5.

nuisance parameter estimated from the data as $\hat{p}_i = n_{i\cdot}/n\,..$, and then $\hat{p}_i$ is substituted for p_i in the log-likelihood function, and the only unknown parameter θ is estimated by using the Newton method. By contrast, we directly maximize the negative log-likelihood function and estimate $\Sigma_{j=1}^{J}(k_j - 1) + 1$ parameters by using a simplex minimization. We estimated the hyperparameters and the dispersion parameter from the mtDNA haplotype distribution among islands for Channel Island foxes given in Table 2 of Rannala and Hartigan (1996) using the full-likelihood function (Equation 3). The estimates were similar to Rannala and Hartigan's PMLE (Table 10). Thus, our empirical Bayes procedure also offers the maximum-likelihood estimators (MLEs) of the rate of gene flow. MLE is more efficient than PMLE (Chuang and Cox 1985) and has the advantage that it can estimate the confidence interval of the parameters by using the likelihood-ratio test.

Wright (1969) proposed the estimator of θ for a discrete-generation island model of a population at equilibrium, based on F_{ST} as $\hat{\theta} = 1/F_{ST} - 1$ (Rannala and Hartigan 1996). Substituting this estimator into Equation 4, we have Equation 13, which was obtained from the total variance of Weir (1996). As is clear from Equation 6, larger σ^2 gives smaller θ, indicating that larger genetic differentiation corresponds to smaller gene flow. For the case of the red sea bream, σ^2 and θ were estimated at 1.80 and 106.55, respectively. For the case of the foxes, they were estimated at 17.89 and 0.45, respectively. From this result, it is clear that the six fox populations in the isolated islands had small gene flow and large genetic differentiation. On the other hand, red sea bream had large gene flow and small genetic differentiation. The estimate of F_{ST} for red sea bream was $\widehat{F_{ST}} = (1.80 - 1)/(87.5 - 1) = 0.0093$, which was relatively small. But for foxes it was $\widehat{F_{ST}} = (17.89 - 1)/(25.5 - 1) = 0.6894$, suggesting advanced inbreeding in the fox populations.

The essential idea for estimating overdispersion is to compare the variation of sample allele frequencies obtained from the different locations to the multinomial variance. In addition, the effective population size is based on the changes in allele frequencies between generations. Conversely, overdispersion provides insight into the spatial variation of allele frequencies. By evaluating the spatial variation, it might become possible to discriminate the overdispersion resulting from the variation between generations. Hence, the procedure needs to evaluate overdispersion as a function of the spatial variation and then measure the variation between generations taking overdispersion into account.

In the three case studies we looked at for estimating the effective population size, direct information on the spatial variation was scarce. Therefore, the precision of the dispersion parameter was marginal. When subpopulations exist, overdispersion arises and affects the estimation of the effective population size. It is then important to collect data on the spatial variation. At the same time, when many isolated subpopulations exist, the effective population size is considered to be close to the size of a subpopulation. When this occurs, it seems dangerous to dismiss the variation between generations as overdispersion. It needs further consideration.

Practical considerations on estimating N_e: From the approximate variance formula of N_e estimate (Pollak 1983, Equations 28 and 29; Waples 1989, Equation 17), it is clear that increasing the sample size, the number of loci, and the number of generations t simultaneously ensures greater precision for the estimate of N_e (Waples 1989). Miller and Kapuscinski (1997) stated that if N_e is expected to be moderately large, the sample size, the number of loci, and the number of generations should all be as large as possible. To improve the precision of the estimate of N_e, it is essential to reduce the sampling variance and increase information on genetic drift.

Sample size: The idea of the temporal method is to estimate N_e from the genetic change over time described by F-statistics estimated from the sample allele frequencies. F-statistics, then, consist of the genetic drift and the sampling variance. To evaluate the genetic drift, we have to subtract the sampling variances from the F-statistics. The second and third terms in the denominator of Equation 10 are the sampling variances at generations 0 and t. If N_e is large, the genetic drift may be small, so the denominator of Equation 10 would take a negative value, which leads to an infinite N_e for small sample sizes n_0 and n_t. If overdispersion arises, the effect of subtracting the sampling variances becomes σ^2 times larger, which is why we failed to estimate the upper limit of the credibility region of N_e. As pointed out by Waples (1989), the temporal method should be useful for cases of small N_e, where larger genetic drift is expected. Even in the case of a small N_e, the problem of an infinite N_e estimate can occur due to large sampling variance, as shown in the ayu studies, because of the small sample sizes. When one uses the temporal method, reducing the sampling variance is indispensable. The sample size should be kept as large as possible. A larger sample size also provides greater information on the genetic drift.

Number of loci: Williamson and Slatkin (1999) developed a maximum-likelihood temporal method to estimate N_e and compared estimates with those derived with the F-statistic method. The simulation result in their Table 1 showed that increasing the number of loci reduced the variance and bias in both estimators, although when the number of loci was >50, the corresponding reduction of variance and bias was not large, and the total information on allele frequency changes did not increase much. The results of Williamson and Slatkin (1999) suggest that information on genetic drift was not improved much even if the number of loci was

>100, because their simulation was based on diallelic alleles. *F*-statistics measure a magnitude of changes in allele frequencies per allele, which can be regarded as a sample mean. So, the estimation precision can be improved if the number of loci is increased. This suggests that increasing the number of alleles is essential, which can be attained by increasing the number of loci.

Number of years between samples: The number of years between samples is correlated with the number of generations, and it then affects the precision of the estimate of N_e. A large number of generations between samples can improve the precision of the estimate of N_e (Waples 1991), because information on genetic drift increases as the number of generations increases. Williamson and Slatkin (1999, Table 1) showed through simulated populations sampled at generations 0–4 and 0–8 that the variance and bias in both estimators were reduced when the number of years between samples was doubled, although the effect of reducing the bias was not clearly observed with the *F*-statistic method.

For the case study of ayu, the posterior mean of N_e was 350 based on F_1 and 796 based on F_3, and SDs for the two estimates were 4024 and 18,538, respectively, showing the reduction of precision despite the fact that the number of years between samples was doubled (Table 8). This is because the doubled number of generations increased the variance of allele frequency changes. The numbers of infinite N_e estimates in 10,000 simulations were 8453 on F_1 and 4927 on F_3, and the smaller F values increased the estimated value of N_e. The result was similar for northern pike. The point estimate of N_e based on F_3 (= 125) was larger than those based on F_1 (= 35) and F_2 (= 72), and the confidence interval for F_1 was the largest (Miller and Kapuscinski 1997).

Miller and Kapuscinski (1997) discussed the question of which estimate obtained from F_3 and F_{mean} to use for the entire time interval. If N_e changes between the two sampling intervals, it should be evaluated by using F_1 and F_2 for the respective intervals. The numbers of adult ayu used for the artificial fertilization in 1997 were 600 females and 480 males, which are lower numbers than those used in 1996. The posterior mean of F_2 was larger than that of F_1, which resulted in a smaller N_e estimate based on F_2, supporting the fact that N_e actually changed (Table 8 and Figure 6). One can use the estimate of N_e based on F_{mean} as the harmonic mean of the effective population size in the respective intervals. The precision was improved by using F_{mean}, in which a larger quantity of information was included. The greater precision that occurred with using F_{mean} and the lower precision that occurred with using F_3 for the case study of ayu are shown clearly in Figure 6.

When N_e does not change in the entire interval, F_3 is expected to have more information on genetic drift than F_1 and F_2. Williamson and Slatkin (1999, Table 1) also showed by their simulation that the estimates of N_e based on F_{mean} had smaller variances and biases than those based on F_1 and F_3. This suggests that the decision about which estimate obtained from F_3 and F_{mean} to use should be made on the basis of the relative effect of improving precision by using F_{mean} and a doubled number of years. Which estimate has more information on the genetic drift must be determined on a case-by-case basis.

Overlapping generations: The basic theory of the temporal method assumes generations to be discrete. The expected number of generations used in Equation 10 directly affects the estimate of N_e. We take time to be measured in years. The expected number of generations between samples can be estimated by dividing the number of years between samples by the mean generation time, which corresponds to the mean age of maturity. In the case of ayu, since the life span is 1 year, 1 year coincides with one generation, which makes it possible to estimate $E[t]$ by the above-mentioned method.

In the case of herring and salmon, however, where there are overlapping year classes of spawners, the estimation of $E[t]$ is complex. When generations overlap, the age-specific birth rate may essentially affect the estimate of $E[t]$. Hill (1979) showed that estimates of N_e are robust with overlapping generations if demographic parameters are stable. If demographic parameters change over time, $\hat{F}$ may be biased upward, leading to an estimate of N_e that is too small (Pollak 1983; Waples 1989). Jorde and Ryman (1995) proposed a correction method for the bias and showed by using simulations for short time intervals that the bias was larger for a case where age-specific birth rates were extremely different compared with a case with equal age-specific birth rates. Waples (1990) developed a statistical method for this situation that can be applied to Pacific salmon populations that have an unusual life history of semelparity with overlapping year classes. Tajima (1992) developed a formula to estimate the expected number of generations without computer simulations and showed the estimates agreed well with estimates obtained by the method of Waples (1990), which requires computer simulations.

In the case of herring, age-specific survival and birth rates were unknown, so it was not possible to apply the method of Jorde and Ryman (1995), which requires these demographic parameters. If an individual continues to spawn every year after the first spawning, like herring, $E[t]$ may be estimated by dividing the years between samples by the mean age of spawners, leading to the estimate of $E[t]$ at 1.48 (= 3/2.1). If a distribution of age-specific birth rates is concentrated to a specific age, $E[t]$ may be close to the estimate obtained by the methods of Waples (1990) or Tajima (1992). We estimated the number of steps at 12 and the expected number of generations at 5.71 (= 12/2.1) for a time interval of 3 yr between samples by using the computer program given in Tajima (1992), where we substituted $f(2) = 0.9$, $f(3) = 0.1$, and $f(4) = 0$. The downward bias

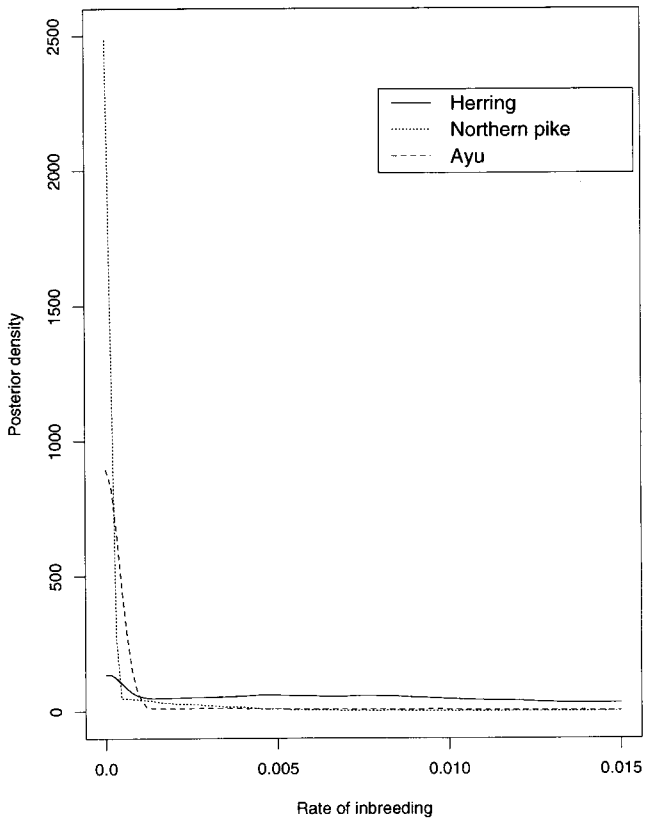

FIGURE 7.—Posterior distributions of the rate of inbreeding of herring, ayu broodstock, and northern pike for the 95% lower limit of the dispersion parameter.

of $\hat{N}_e$ when demographic parameters change over time with overlapping generations should be corrected upward. From a conservation viewpoint, the estimate of N_e without the correction must be conservative for the overlapping generations.

Rate of inbreeding: As another evaluation of breeding population size, the inbreeding coefficient may be useful, especially for cases where the population size is estimable, as it is in the field of fishery science. CROW (1954) used the inbreeding effective size, making a distinction between that and the variance effective size, which was defined by an inverse of the inbreeding coefficient. However, it is known that there is no great difference between the two effective sizes (NEI 1987); hence we calculated the posterior distribution of the rate of inbreeding defined as $1/(2N_e)$ (FALCONER and MACKAY 1996), as an infinite N_e corresponds to an inbreeding coefficient of 0, obtained from the 95% credibility region. The means, SDs, and 95% credibility regions of the posterior distributions of the rate of inbreeding were 0.0083, 0.0070, and [0, 0.0251] for herring; 0.0004, 0.0012, and [0, 0.0041] for northern pike; and 0.0011, 0.0041, and [0, 0.0140] for ayu.

The posterior mean of herring was 7.5 times larger than that of ayu with a right-tailed credibility region shown in Figure 7. Overdispersion for herring was underestimated, because the samples for males and females taken in 1996 were analyzed as independent samples even though they were the same sample, leading to a smaller value of N_e, which caused the rate of inbreeding to be overestimated. The mean for northern pike was smallest with the narrowest credibility region. However, these values may be underestimated because of an overestimated dispersion parameter of northern pike, which was the largest among our four case studies. There was only one sample for one sampling year, and we assumed that the seven loci had common hyperparameters, so the estimated dispersion parameter may include the change of the allele frequencies.

Multistage sampling in hatcheries: All existing methods assume that N_e is drawn from a gamete pool by a simple random sampling. This is an appropriate assumption for the reproduction of a wild population. However, for broodstocks cultured over generations in hatcheries, candidates of the next broodstock are sampled from the progenies produced by the broodstock. Therefore, N_e is drawn from the progenies by a two-stage sampling. If artificial fertilization using a part of the candidates is performed, as in the case study of ayu, N_e is drawn from the progenies by a three-stage sampling and the sample is drawn from the candidates to estimate the allele frequencies, which is therefore a two-stage sampling of the progenies. The multistage sampling must lead to the different form of $V(x - y)$ given in WAPLES (1989). This is a problem that needs further research, but it should be noted that the variances corresponding to the two-stage and three-stage sampling become small when the sample sizes are large. In the case of ayu, a total of 3000–4000 candidates were sampled from the progenies and cultured in rearing tanks, and 1500 adult fish from the candidates were used for artificial fertilization. Hence the sample allele frequencies of ayu were expected to represent those of the progenies produced by the broodstock. However, if the sample sizes are small, $V(x - y)$ is seriously affected.

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APPENDIX

Expectation of $\sqrt{X}$ when X follows a χ^2 distribution: Let X be a random variable that follows a χ^2 distribution with degree of freedom of k. We derive here the expectation of $\sqrt{X}$. Generally, when X is continuous and has a probability density function $f(x)$, the expectation of $g(X)$ is given by

$$E[g(X)] = \int g(x) f(x)\, dx.$$

For our case, the expectation of $\sqrt{X}$ is calculated as

$$E[\sqrt{X}] = \int \sqrt{X} f(x)\, dx = \frac{1}{2^{k/2}\Gamma(k/2)} \int x^{(k+1)/2-1} e^{-x/2} dx.$$

Let $x/2 = y$, and we have

$$E[\sqrt{X}] = \frac{1}{2^{k/2}\Gamma(k/2)} \int (2y)^{(k+1)/2-1} e^{-y} 2\, dy.$$

Using the gamma function, which is given by

$$\int y^{(k+1)/2-1} e^{-y} dy = \Gamma\left(\frac{k+1}{2}\right),$$

finally we have

$$E(\sqrt{X}) = \sqrt{2}\, \frac{\Gamma((k+1)/2)}{\Gamma(k/2)}.$$